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of the swing of the pendulum; the pin is brought in contact with the plate, closing an electric circuit, which actuates a time-marker writing upon the recording surface. As the lever rebounds, and does not make contact again until it has received another impulse, the electric closure is almost instantaneous.

The great advantage of this simple arrangement is that it does not involve any alteration in the apparatus with which it is used. A cork disc or ball slipped upon the end of the vibrating rod is the only addition to the metronome that is required. The disadvantages of a mercurial contact are avoided, which is always desirable, unless special reasons require it.

The apparatus has been tested with a recording tuning fork and it has been found to give satisfactory results with the metronome, which, of course, should only be used in experiments of moderate accuracy. The key is probably much more reliable than the metronome with which it is used. Comparative tests of the key and the well-known tambour device for recording the beats of a metronome were also made, and the key was found much more convenient and accurate.

I have ventured to describe this inexpensive piece of apparatus, as its simplicity and efficiency would seem to commend it to all those who employ the metronome in recording time.

FREDERICK W. ELLIS

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SOCIETY OF AMERICAN BACTERIOLOGISTS

V

Industrial Bacteriology

Under the supervision of R. E. Buchanan

Problems in Soil Bacteriology: JACOB G. LIPMAN.

The student of soil bacteria, and of other soil microorganisms, is often struck by the fact that there is, apparently, localization of one or another of the species in certain spots. To what extent is this localization characteristic of fields, small areas in any one field, or soil particles of different mineralogical or other origin? We know, of course, that the water films surrounding the individual soil particles represent solutions of varying composition and concentration. But we have no knowl-

edge, except of an indirect character, as to the very interesting differences which must exist as to the numbers and kinds of bacteria in the water film surrounding individual particles.

From other fields of bacteriology, we know that there may be associative action, and likewise antagonism, among species of soil bacteria. But we know practically nothing of these relations in the soil, nor how these relations may be modified by soil treatment or by climatic conditions.

It has always been my belief that the beneficial results ascribed to applications of commercial fertilizers or of other materials may be due as much to the action of such materials on soil microorganisms as to the action on the crops themselves. There is need now for the study of soil bacteriological problems from this point of view.

Another problem which is widely recognized of importance to soil fertility is the formation of so-called humus in the soil. Admitting that humus is the result of biological activities, it is for us to discover how the composition of the resulting product is affected, not only by vegetable and animal substances from which it is derived, but by the type of microorganisms concerned in its formation.

To the problems already mentioned, I might add the systematic study of temperature, moisture, aeration and pressure as factors in influencing bacteriological activities in the soil. It is possible also, that so-called stimulants, like manganese, copper, zinc, etc., may react on the activities of soil microorganisms. These problems should receive the attention of, preferably, a large number of workers. It may be added that these and other problems studied systematically will help to throw light on the production and modification of plant food in the soil and on the great industry of crop production.

The Solution Versus the Soil Method for the Bacteriological Examination of Soils: P. E. BROWN.

From a careful study of the methods which have been employed for the bacteriological examination of soils, it is concluded that the "fresh soil" method is the most rational which has yet been devised. A recently proposed modification of the solution method, while eliminating some of the objections to the old method, is considered to possess many objectionable features, so many as to be of very questionable value for the interpreting of results from the fertility standpoint. It is urged that careful comparative tests be carried out, in order to settle definitely the question of which is the "best" method for the bacteriological examination of soils.

Relation of Lime to Production of Nitrates and Mineral Nitrogen: F. M. SCALES.

The lime requirement of an acid soil was determined by adding varying quantities of calcium carbonate to weighed portions of the soil, moistening and, after an hour, testing with litmus paper until a quantity was found that gave a neutral reaction. The lime requirement by the Veitch method was the same as the above. Fractions and multiples of this requirement were added to 100 gm. portions of soil which received in addition for one duplicate set ammonium sulphate and for another duplicate set alfalfa powder. They were moistened with 18 per cent. of distilled water and incubated for three weeks at 28° to 30° C. Determinations of nitrate and mineral nitrogen present in the samples showed that the nitrifying bacteria were most active in the presence of 50 per cent. of the calcium carbonate requirement and the ammonifying and nitrifying groups combined in the presence of 75 per cent. of the amount required according to the chemical determinations. In this particular soil an excess of calcium carbonate was markedly toxic for the nitrifying organisms and not stimulating for the ammonifiers. Some common crop plants are to be grown on this and other soils containing varying quantities of lime to determine what relation exists between the lime requirement for optimum nitrification and for ammonification and nitrification combined and that for the best growth of the plants.

A Soil Sampler for Soil Bacteriologists: H. A. NOYES.

The object of this sampler is to furnish a piece of apparatus which will sample the soil under one system of cultivation as well as under another. It also becomes the container for the soil after the sample is taken.

The sampler is a brass tube 11 inches long, with one end made into a cutting edge. This cutting edge is so made that the soil is not appreciably compacted when the sample is taken. The end having the cutting edge is furnished with a tight-fitting brass cap two inches in height. The open end, plugged with absorbent cotton, makes the sampler complete. The procedure in using this apparatus follows: Plug and cap as many samplers as you wish to take samples of soil; sterilize them in the hot air sterilizer and take them to the field. Remove a cap from a sampler, insert the driving head above the cotton plug and drive the sampler into the ground to the desired depth, pull it out, flame and return the cap and the sample is ready to take to the laboratory.

The sampler has the following properties which are important in bacteriological work: Easily sterilized; easily kept clean; easily manipulated; durable.

The Effect of Phosphates and Sulphates on Soil Bacteria: E. B. FRED.

The influence of inorganic fertilizers on the bacterial processes of the soil has not received much attention. For this reason a study of the effect of some of the pure salts of those elements which constitute an important part of commercial fertilizers was undertaken.

The aim was to determine, if possible, the influence of phosphates and sulphates upon the activities of soil bacteria and determine if the fertilizing effect of these substances could be explained in part by the promotion of bacterial action.

The following methods were employed:

Rate of ammonification in solution and in soil; this was conducted with pure and with mixed cultures of bacteria. Aside from this, determinations were made of the relation of the number of cells to the amount of nitrogen ammonified. To show this relation, plate counts were used. The nitrogen for ammonification was added to the solution in the form of peptone and to soil in the form of casein. The rate at which the nitrogen of these substances is converted into ammonia, was determined by distilling with magnesium oxide. The cultures were incubated at room temperature and at different intervals the amount of ammonia determined.

Monobasic potassium phosphate in peptone solution caused a great increase in the production of ammonia. This is noted with a pure-culture yellow ammonifier and with a suspension of soil bacteria. The gain was greatest at the end of the first two days.

Merck's precipitated calcium phosphate caused a slight increase in ammonification, but not nearly so large as the monobasic potassium phosphate.

Sulphates of calcium and potassium increased ammonification to a small extent.

The action of monobasic potassium phosphate was far greater than that of potassium sulphate. From this it seems that the potassium ion does not materially influence ammonification.

The results of plate counts show that monobasic potassium phosphate causes an enormous increase in multiplication of bacteria. This is followed by a rise in ammonia. The ammonia production, however, is not in proportion to the number of bac-

teria. This seems rather to be a result of increase in the number of cells than increase in individual cell activity.

All of the phosphates gave a large increase in the number of soil bacteria. There was only a slight increase from the sulphates.

The same relative effect of phosphates and sulphates was noted in the case of carbon-dioxide evolution.

From the results of this work, as a whole, the following conclusion may be drawn:

That possibly the increased crop production which results from the application of soluble phosphates is due in part to the promotion of bacterial activity.

The details will appear in a future publication.

The Effect of Green Manures on the Germination of Various Seed: E. B. FRED.

When green manures are turned under and the soil planted immediately, a decrease in germination may result.⁶

This problem was considered of sufficient importance to warrant a series of field and laboratory experiments in an endeavor to find some explanation for this phenomenon. The causes that might be offered to account for the harmful influence of green manures on seed germination are:

First, that the green manure not only causes a marked increase in number of bacteria, but also a change in the flora.

Second, that the great increase in number of bacteria results in a possible accumulation of some substance or substances, toxic to germination.

Third, that the rapid multiplication of microorganisms greatly increases their metabolism.

In order to gain some idea of the practical importance of this problem, a series of field tests was conducted. The results of this work show that when green clover or oat tissue is turned under and the land planted immediately, there is a distinct decrease in the rate of germination with cotton, soy bean and hemp seed. The cereals, corn and oats fail to show any injury from green manures. After twenty-five days the injurious factor seems to have disappeared entirely.

Under greenhouse conditions it has been found that small amounts—0.25 per cent.—of green manures are injurious to the germination of cotton seed. Larger amounts are more effective.

The addition of calcium carbonate to the green manure fails to prevent the injurious action.

The degree of retardation seems to vary somewhat with the soil type; in heavy soils green ma-

⁶ Hoffman, Exp. Sta. Bull. 228, 1913, p. 26.

nures have their most marked effect; furthermore an increase in moisture causes a decrease in rate of germination.

When peptone and casein are added in the same nitrogen ratio as the green manure, no decrease in germination is noted. Soluble carbohydrates in amounts of 1 to 2 per cent. retarded the rate of germination, but did not cause the seed to decay as in the case of green manures.

Determinations of carbon dioxide and ammonia in green manure soils were made. Periodic analyses failed to show the presence of these in quantities great enough to account for the injury to seed germination.

A more complete report of this work will appear in bulletin form.

Standard Methods of Bacteriological Analysis of Milk: H. W. CONN.

Professor Conn gave an account of an extended series of cooperative experiments in four laboratories in New York upon the reliability of the bacteriological examination of milk, and as a result of the facts that were brought out by the cooperative tests, reported that the Committee on Standard Methods of the American Public Health Association had made the following changes in methods of milk analysis.

First, that beef extract (Liebig) be substituted for beef infusion in the making of agar media.

Second, that 1.2 per cent. dry agar or 1.5 per cent. ordinary moist agar be the amount used in standard medium.

Third, the acidity of standard medium shall be 1 per cent.

Fourth, all plates shall be incubated at 37½ degrees for 48 hours before counting.

Fifth, plates shall be counted with a magnifying power of 3½ diameters.

The Alkali-forming Bacteria Found in Milk: S. HENRY AYERS AND PHILIP RUPP.

The alkali-forming bacteria may be broadly defined as those which produce an alkaline reaction in milk within 7 days, due to the oxidation of salts of organic acids, which results in the formation of alkali carbonates. No visible sign of peptonization is produced.

Probably all the alkali-forming bacteria produce ammonia upon long inoculation, but the preliminary alkaline reaction is due to the production of alkali carbonates and not to ammonia. The presence of alkali carbonates in milk can be determined by the addition of casein dissolved in sodium phosphate.

Alkali-forming bacteria are very common in milk, but would rarely be noticed on litmus-lactose agar plates. They can be found by inoculating into tubes of litmus milk and observing the reaction after 7 to 14 days' incubation at 30° C.

The alkali-forming bacteria can obtain their nitrogen from meat juices, peptone, casein, gelatin, and many can also, with few exceptions, use nitrogen from inorganic salts, such as sodium ammonium phosphate and probably all ammonium salts; also from sodium nitrate and nitrite.

The best source of carbon seems to be the salts of organic acids.

Since various cultures ferment salts of different organic acids, we hope to be able to classify this group of bacteria on these fermentations. In sugar broths only an alkaline reaction is produced and consequently the sugar fermentation is of no value as a means of classification.

We believe the fermentation of salts of organic acids will be of great value in the classification of bacteria, particularly soil bacteria, which do not ferment sugars when present in broth.

Decomposition of Casein in Presence of Salt by Butter Flora: CHAS. W. BROWN.

The casein in butter during storage is slowly broken down into amino-acids and ammonia. Nitrogen, as amino-acids and ammonia, in percentage of the total nitrogen in unsalted butter (average of tubs from three creameries) was found to be 5.71 per cent. at first and 7.59 per cent. after 240 days' storage at 21° F.; in salted butter from the same three churnings and stored in the same storage was found 5.71 per cent. at first and 8.19 per cent. after 240 days. And again, in salted butter made from pasteurized and from unpasteurized cream, the percentage (average of 20 tubs) increased from 6.24 to 6.86 per cent. for the pasteurized and from 7.68 per cent. to 8.25 per cent. for the unpasteurized during storage at 0° F. for 428 days.

Pure cultures of twelve different bacteria isolated from storage butter when introduced separately into flasks of sterile separated milk and also into other flasks of the same milk to which was added 5 per cent. sterile salt and incubated at 20° C., caused a decomposition of the casein during 1, 3 and 7 days as follows: The nitrogen (per cent. of total milk) found as caseoses and caseones (average for 12 different bacteria) was 0.031 per cent., 0.037 per cent. and 0.054 per cent. in plain milk; and 0.030 per cent., 0.034 per cent. and 0.042 per cent. in milk with 5 per cent. salt;

the nitrogen found as amino-acids and ammonia was 0.031 per cent., 0.042 per cent. and 0.076 per cent. for plain milk and 0.028 per cent., 0.035 per cent. and 0.041 per cent. for salted milk.

While the activities of butter flora in the decomposition of casein milk with or without salt can not be considered to parallel their action in butter, yet can we not assume safely that at least part of the casein decomposition in butter is due to the butter flora?

The Presence of Streptococci in the Milk of Normal Animals: J. M. SHERMAN AND E. G. HASTINGS.

In many public-health laboratories the routine examination of milk includes tests for streptococci. The supposed relation between udder streptococci and septic sore throat in man is the reason for making such examinations.

The examination of the milk from 88 individual animals in four herds demonstrated the presence of streptococci in 38.6 per cent. of the samples of milk. The animals were all free from udder trouble. The examination of the product of twelve herds demonstrated the presence of streptococci in the milk of ten of the twelve examined. In all of the above cases 1/100 c.c. of milk was used.

It would seem that the milk of healthy animals frequently contains streptococci at the time it is drawn from the udder, and that before much emphasis can be placed on the detection of these organisms in milk, methods by which harmful types can be differentiated from the harmless ones must be devised.

The milk of most of the herds examined was used chiefly for the feeding of children. No known cases of trouble have resulted.

The Refrigeration of a City's Milk Supply: CARLETON BATES.

This paper sets forth the plan of a milk campaign as conducted by the Bureau of Chemistry, U. S. Department of Agriculture. It further sets forth the results of the bacteriological examinations of a city's supply, the causes of the high bacteriological counts obtained, and means employed for remedying the causes.

The chief cause of the high bacteriological counts was due to non-refrigeration of milk in transit, the average temperature of the milk upon receipt in the city being about 65° F. This milk was en route from six to twelve hours.

After refrigeration had been provided by the railroads the milk, at the present time, is being received in the city at about 48° F.

Slimy and Ropy Milk: R. E. BUCHANAN AND B. W. HAMMER.

A study of slimy and ropy milk sent for examination to the Dairy Bacteriological Laboratories of Iowa State College has shown the following:

1. Cultures of organisms secured from slimy starters, apparently typical *Streptococcus lacticus* forms, sometimes showed marked capacity to produce ropiness when inoculated into sterile milk.

2. Associative action of organisms in some cases is responsible for ropiness.

3. *Bacterium (lactis) viscosum* is one common cause of slimy milk.

4. Certain peptonizing bacteria, as *Bact. peptonogenes* produce a very slimy residuum after digestion of the casein.

5. *Bacterium bulgaricum* and certain related high acid organisms frequently produce marked viscosity in milk.

Sliminess in milk, therefore, is apparently due to different causes with different organisms.

Methods of control and prevention of slimy milk are discussed.

Descriptions of thirty-three species of bacteria that have been found associated with milk are given, and the literature reviewed.

Factors Influencing the Resistance of Lactic Acid Bacteria to Pasteurization: K. PEISER.

In milk and cream pasteurized at 63° C. (145° F.) for twenty minutes in a "Perfection" Pasteurizer (200 gal. capacity) were found a number of strains of the *Bact. lactis acidii* type whose thermal death-point in broth is below the pasteurization temperature.

The thermal death-point of a number of these strains was determined in bouillon (10° acid to phenolphthalein) and in boiled whole milk, separated milk and milk serum, with the result that the average thermal death-point is in whole milk 5° C., in separated milk 2.5° C., and in whey 0.5° C., higher than in bouillon. These results indicate that the protection given to the suspended lactic bacteria by the casein and coagulated albumen of separated milk raised their thermal death-point 2.5° C. and that the protein and fat of whole milk raises their thermal death-point 5° C. In this we see a reason why some bacteria whose thermal death-point is low are found in pasteurized milk.

Bacteria in Preserved Eggs: MAUD MASON OBST.

Commercial and strictly fresh June eggs packed in solutions of 1:5, 1:10, 1:15, 1:20 parts commercial waterglass, and in saturated lime solutions

were stored in laboratory, barn, cellar and at 34° F. Thermograph records were kept. Bacteriological and chemical examinations were made, also cooking experiments and parcel-post shipments.

Temperature of 80° F. in laboratory permitted rapid multiplication of bacteria in eggs.

Barn temperature varied from 10° F. to 87° F. Eggs froze in solutions, later some thawed without breaking and at end of experiment showed no effects attributable to freezing. Bacterial content was uniform and fairly low. Bacterial increase in commercial eggs in 1:10 waterglass was rapid, especially in albumen, during first two months of storage.

Eggs stored in cellar held a uniformly low bacterial content throughout experiment.

At 34° F. eggs showed exceptionally low counts.

Waterglass solutions contained practically no bacteria per c.c. after five months of storage. Average bacterial content of eggs in nearly every lime solution increased more rapidly than in waterglass, necessitating the discard of certain lime solutions early in experiment.

Curves were plotted showing increase of average bacterial content in relation to length of storage. Bacterial content of albumen in most cases remained lower or equal to that of yolks for 150 or 250 days of storage, then the former increased markedly and generally far exceeded that of the yolk.

From good eggs were isolated: *M. aurantiacus*, *B. prodigiosus*, *B. subtilis*, *B. pyocyaneus*, *B. fluorescens liquefaciens*, *B. termo*, *B. zopfii*. One decomposed egg contained *B. proteus* in large numbers.

Some Methods and Appliances Used in the Elementary Courses in Bacteriology: W. H. WRIGHT AND E. G. HASTINGS.

A description of the laboratory equipment used with large classes.

The Effect of Certain Organic Soil Constituents on the Fixation of Nitrogen by Azotobacter: BRUCE WILLIAMS.

This paper reports a study on the effect of various organic compounds on the growth of *Azotobacter*. The compounds used were those likely to be constituents of the soil.

One liter Erlenmeyer flasks, to which were added 15 grams of pure sea sand, previously washed and burned, afforded an excellent surface upon which *Azotobacter* developed. To each of these flasks was added 100 c.c. of Ashby's media.

The flasks were sterilized under 15 pounds of steam pressure for 15 minutes. After this sterilization, the compounds were introduced into the flasks in desired concentrations and all flasks received equal inoculation of pure cultures of *Azotobacter* previously grown on Ashby's agar and suspended in sterile water. Two flasks were set up for each compound in every concentration and two control flasks receiving only inoculation were used to test the fixation power of the culture used. All flasks were incubated for 21 days, at the end of which time nitrogen determinations of the content of each flask were made by the Kjeldahl method.

In studying compounds which contain nitrogen four flasks instead of two were set up with each compound, two of the flasks receiving inoculation with *Azotobacter* and the remaining two used as controls for the nitrogen content of the compound—these latter flasks were kept in the incubator room during the period of incubation.

The concentrations employed were on the basis of p.p. Mil. or 0.025, 0.05, 0.1 and 0.2 gram per liter.

The results of the study indicate that fixation of nitrogen by *Azotobacter* is only slightly influenced by most of the compounds investigated.

Hydroquinone and salicylic aldehyde revealed the most toxic properties of any compounds studied.

Esculin, quinic acid and borneol afforded marked stimulation to the growth of the organism.

The effects of the compounds on *Azotobacter* are not, as a rule, in accord with what has been reported of their action on the higher plants. In concentrations which are fatal to certain higher plants, many of the compounds only slightly depressed fixation.

Such compounds as nicotine, picoline, guanidine and skatol exhibited toxic properties commensurate to those usually ascribed to these substances. Caffeine appeared to stimulate the growth of the organism.

Many of the nitrogenous compounds used which have been reported as beneficial to higher plants exercised a marked depression on fixation. It appears that the simpler compounds were more pronounced in this respect than were the more complex ones. It is suggested that this condition is not one of toxicity, but that the nitrogen of the compounds was utilized by *Azotobacter* in preference to that of the atmosphere. Urea, glycocoll, formamide and allantoin were especially active in depressing fixation.

Relation of Numbers of Streptococcus lacticus to Amount of Acid Formed in Milk and Cream:
P. G. HEINEMANN.

Erlenmeyer flasks were filled with 250 c.c. each of milk and cream. Three flasks of each series were sterilized and then inoculated with a culture of *Str. lacticus* in litmus milk. Three flasks of raw milk and cream were also inoculated. Three flasks of each were left to sour spontaneously. The flasks were incubated at three different temperatures, 37°, 20° and 7°. Plates were prepared from the original milk or cream and the number of bacteria counted. The acidity was determined by titration with one twentieth normal sodium hydrate phenolphthalein as indicator. Every day for ten days the milk was titrated and counts made by plating. The determinations were made with the cream for eight days.

The following facts were observed:

1. The amount of acid formed during the souring process of milk or cream is not solely dependent upon the number of bacteria present of the *Str. lacticus* group. Temperature and the presence of other bacteria may influence the result.

2. In raw milk or cream or in raw milk or cream inoculated with cultures of *Str. lacticus* the number of bacteria increases to a given point and then decreases. The higher the temperature up to 37° C. the earlier is the maximum number reached.

3. Coagulation of milk or cream is not dependent solely upon a certain amount of acid or a certain number of bacteria.

4. After the decline in numbers the amount of acid continues to increase, probably due to enzyme action.

5. At 37° extraordinarily large amounts of acid may be formed, due probably to the presence of members of the group of lacto-bacilli.

The Variability of Two Strains of Streptococcus Lacticus: P. G. HEINEMANN.

The present investigation was conducted to determine the possibility of varying the fermentative power of *Str. lacticus* by animal passage. Two strains were isolated and inoculated into rabbits and guinea-pigs. The amount of acid produced by the original culture was determined by titration after three days' incubation at 37° C. After each passage the recovered organism was again inoculated into the solutions of test substances and the acid determined again. The amount of available free oxygen was regulated by filling nessler tubes with definite amounts of the

test solutions. The test substances used were dextrose, lactose, saccharose, raffinose, inulin, salicin and mannit.

The main conclusions reached by the work are:

1. The power to hemolyze human and goat's blood may be acquired to some extent by animal passage.

2. Animal passage develops and increases virulence of *Str. lacticus*.

3. Virulence develops more rapidly in rabbits than in guinea-pigs.

4. By animal passage the amount of acid produced in the original strain decreases progressively and fermentation of some of the substances is inhibited.

5. Raffinose and inulin, which were not fermented by the original strains, were fermented to a limited degree after animal passage.

6. Presence of free oxygen seems to favor the production of acid. Under anaerobic conditions less acid was produced than with free access of oxygen. Under anaerobic conditions fewer substances were fermented than under aerobic conditions.

Bacterial Infection of Fresh Eggs: DOROTHY W. CALDWELL.

This paper presented the results of a bacteriological study of fresh eggs carried on at the Agricultural Experiment Station of the Rhode Island State College. The results are, briefly, as follows:

1. Of 2,510 fresh eggs from 65 hens, examined by the indirect method, 8.8 per cent. showed infection in the yolk.

2. None of 111 whites examined showed infection, while the yolks of the same eggs gave a percentage of infection (4.5) less than the average for the series (8.8).

3. The percentages of infection obtained for individual hens per year varied between 2.8 and 15.0, the average being 8.0 per cent. per year. No hen laid sterile eggs during a whole year.

4. No correlation was observed between the percentage of infection for any individual and the degree of fecundity of that individual.

5. Approximately the same amount of infection was found among fertile eggs (6.9 per cent. infected out of 422 eggs examined) as among infertile (8.9 per cent. infected out of 315 eggs).

6. The infection of eggs in the degree made apparent by the present studies seemed to have no unfavorable effect upon their hatchability.

7. Practically no difference between the percentages of infection of eggs from pullets and from hens in their second laying year was found.

8. No definite seasonal variation was observed in the bacterial content of the eggs examined.

9. No definite conclusions can be drawn from these studies regarding the influence of temperature upon the detection of infection in fresh eggs.

10. From fifty-seven infected eggs out of 737 examined in one of the series, 37 bacterial types were isolated, among which were seven cocci, eleven motile rods, eighteen non-motile rods and one spirillum.

11. Control plates exposed under the hood in which the examinations were made yielded a variety of organisms, largely chromogens. This series, as a whole, did not resemble the series of egg organisms.

Regarding the source of infection, this study indicated that the penetration of the shell after the egg had been laid, or infections during the passage of the egg through the cloaca, or during fertilization or while the albumen or the shell were being deposited, are, to say the least, uncommon. It seems more likely that infection of fresh eggs is largely due to occasional chance infections with harmless organisms taking place within the ovary of the fowl.

A New Microscopic Test for Pasteurized Milk: W. D. FROST.

This test differs from a similar one described in 1911 by Frost and Ravenel, in the method of applying the stain, the nature of the stain and the principle involved. A few cubic centimeters of milk have mixed with them one fifth as much of a saturated aqueous solution of methylene blue. This colored milk is allowed to stand about 30 minutes; it is then centrifuged and the sediment spread on a glass slide. When dry it is ready for examination.

In raw milk the microscopic field is stained a uniform blue in which appear clear areas which are either fat globules or leucocytes. The polymorphonuclear cells are irregular in outline, about 12 mikrons in diameter and unstained or only slightly tinged. The sediment from milk heated to 60° C. or above presents a very different picture. The polymorphonuclear leucocytes are rounded up and shrunken so that they are only about 8 mikrons in diameter and the nuclei are deeply stained.

The method requires little more time than it does to make a fat determination and is apparently as simple and accurate as the laboratory diagnosis of diphtheria or rabies.

A. PARKER HITCHENS,
Secretary